INSTITUTIONEN FÖR VIRKESLÄRA

*SKOGSHÖGSKOLAN*

DEPARTMENT OF FOREST PRODUCTS

*ROYAL COLLEGE OF FORESTRY*

Rapporter

Research Notes

Nr **R 78** 1972

# Defibration of wood by the use of a white-rot fungus

## *Defibrering av ved med hjälp av en vitrötesvamp*

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STOCKHOLM

1972

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SUMMARY

When investigations were carried out on wood destroying fungi in the Baltic a project on the screening of fungi suitable for defibration of wood was also in progress. A Basidiomycete (P-Bl)with a pronouced capacity to cause defibration in birch wood was then found in the northernmost part of the Baltic Sea.

This fungus, probably a *Peniophora* species attains optimal growth at +35°C and pH 5. Sodium chloride so strongly inhibits growth that the fungus can be described as halophobe.

Cellobios, glucose and mannose were excellent carbon sources for B-Bl. Galactose could not be utilised at all and the growth on xylose and arabinose was fairly slow.

Decay experiments with birch wood showed that the fungus causes rapid wood decomposition. In the incipient stages lignin is attacked to a much greater extent than the cellulose. At a weight loss of 15 percent, no less than 50 percent of the lignin but only 2 percent of the cellulose had been decomposed.

Birch wood chips pre-treated with various nitrogen compounds were inoculated with P-Bl. Microscopical investigations of the attacked chips showed that the type of attack on the wood fibres varied with various pre-treatments. Impregnation with asparagine, for instance, resulted i.a. in heavy delignification and defibration. Ammonium nitrate on the other hand produced only an attack of the erosion type and no delignification.

Mechanical treatment of the attacked chips and fractioned filtration showed that good defibration and a clear tendency towards paper formation (felting) could be obtained after certain pre-treatments (e.g. asparagine). Impregnation with ammonium nitrate gave no defibration and no tendency towards paper formation. By changing the physiological environment in the wodd it was evidently possible to control the type of attack exerted by P-Bl on the wood structure.

## 1. INTRODUCTION

The old idea of using white rot fungi for the delignification of wood was seriously approached by Eriksson and Godell (1972). By inducing mutations they were able to produce cellulase-less mutants of the very active white rot fungus *Polyporus adustus* Willd. ex Fr. Further work is now being done to study the defibration ability of these mutants (Eriksson & Nilsson, personal communications).

During the screening of white rot fungi suitable for mutagenization to yield potential defibrating strains, a white rot fungus was found which already caused defibration of birch wood without genetical manipulation. Some facts concerning the physiology of this fungus and its attack on birch wood are presented in this paper.

## 2. ORIGIN AND ISOLATION OF THE FUNGUS

The white rotting Basidiomycete P-B1 was isolated from a birch wood sample which had been submerged from 25th July to 29th October 1970 near Piteå in the Gulf of Bothnia in the northernmost part of the Baltic Sea. Before submerging, the sample was sterilized in an autoclave resistant envelope which was not removed until the sample had been installed below the water surface. A similar procedure has been used in other places where wood-destrying fungi have been isolated in Swedish coastal waters.

The fungus has yet not been fully identified. However, it probably belongs to the *Peniophora cremea* group.[1)] In checking the ability of the fungus to attack wood, small samples of birch and pine sapwood were placed on test tube cultures of P-B1. After 145 days the samples were taken out for microscopical examination. They then displayed distinct defibration features.

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1) Work on identification of P-B1 is kindly undertaken by prof Dr Aino Käärik, Department of Forest Products, Royal College of Forestry, Sweden.

## 3. SOME PHYSIOLOGICAL DATA

The growth of P-B1 at various temperatures was studied by measuring the daily radial growth of the mycelium on malt agar plates. The optimum temperature for growth was found to be approximately 35°C and the growth rate was unusually high, 27.7 mm/day. See fig 1.

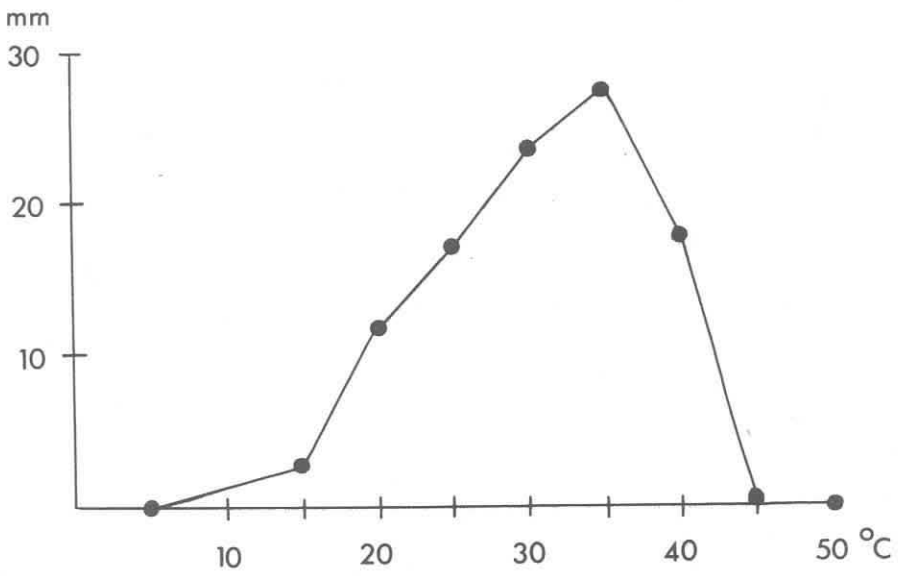


Fig 1. The daily radial growth on malt agar plates of P-B1 at various temperatures.

*Radialtillväxten per dag hos P-B1 växande på maltagar vid olika temperaturer.*

Agar plates with pH values ranging from 1.5 to 8.3 were prepared by the addition of varying amounts of hydrochloric acid and sodium hydroxide into the substrate. The daily radial growth at ca 22°C was measured. Optimal growth was registered at pH 5. See fig 2.

Since P-B1 was isolated from the Baltic Sea where the salinity decreases from the south to the north, variation in the content of sodium chloride in the environment might be a factor of importance for the growth and geographical distribution of that particular organism. The growth of P-B1 on malt agar plates with various NaCl contents was therefore studied. The incubation temperature was 22°C. Fig 3 shows that the fungus is repressed in its growth even at very low NaCl percentages. Consequently, P-B1 is not likely to thrive in marine habitats.

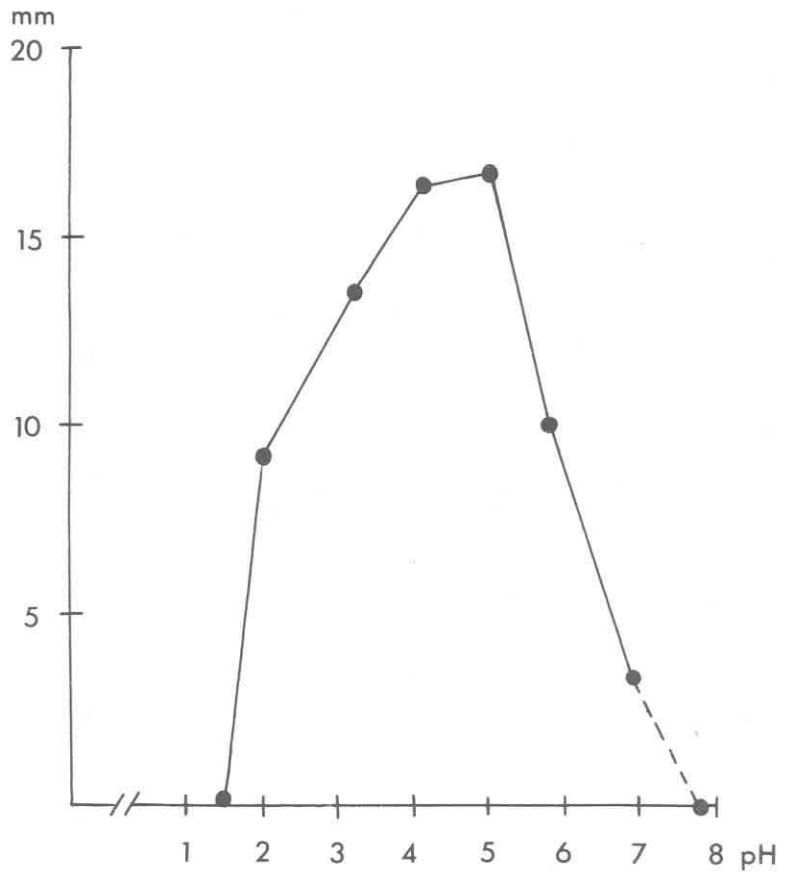


Fig 2. The daily radial growth of P-B1 on malt agar plates with varying substrate pH.

*Radialtillväxten per dag hos P-B1 växande på maltagarplattor med olika substrat-pH.*

The growth of P-B1 on various carbohydrates was studied. For that purpose the fungus was cultivated in 100 ml Erlenmeyer flasks containing 30 ml of a nutrient medium C (Lundström 1970) to which 20 gm/litre of various sugars was added. The flasks were either placed on a reciprocal shaker at 22°C or left standing without shaking at 35°C (optimum temperature for mycelial growth). Small pieces from a malt agar culture of the fungus were used as inocula. As the fungus produces very little of aerial mycelium, the inoculas immediately sank to the bottom of the flasks. Thus, the shake cultures represent a well-oxygenated and the standing cultures a restricted oxygen supply condition. The following mono- and disaccharides were tested: D-arabinose, D-xylose, D-glucose, D-galactose, D-mannose, D-cellobiose.

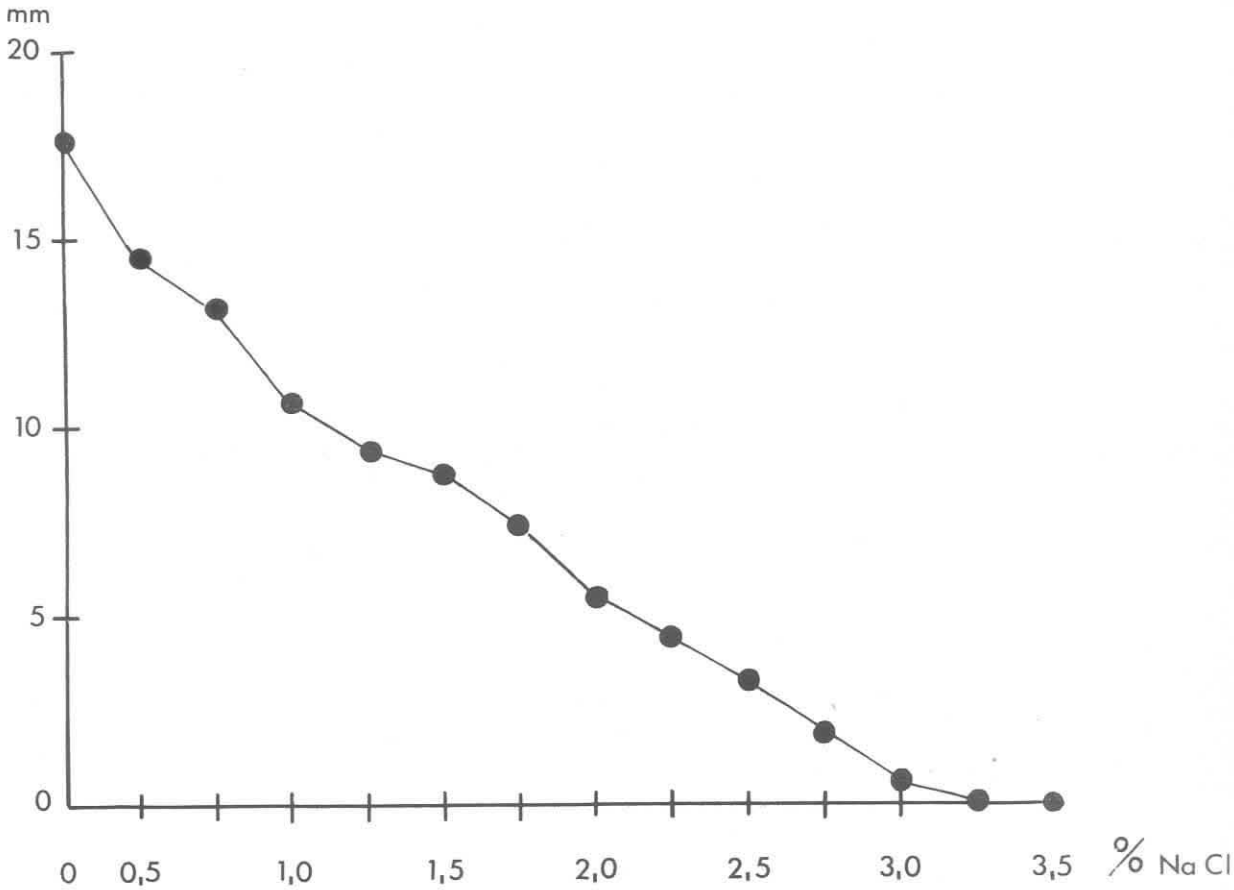


Fig 3. The daily radial growth of P-B1 on malt agar plates containing various amounts of NaCl.

*Radialtillväxten per dag hos P-B1 växande på maltagarplattor innehållande olika mängder NaCl.*

The results are demonstrated in fig 4. The white rot fungus P-B1 readily utilized cellobiose, glucose and mannose when the oxygen supply was good. At restricted oxygen supply the growth on cellobiose was superior to that of glucose and mannose. The utilization of galactose was nearly negligable at both oxygen levels, as well as that of arabinose at restricted oxygen supply. Arabinose was, however, consumed at a slow rate at the higher oxygen level. The growth on xylose was fairly good at both oxygen levels although this pentose was a significantly poorer carbon source than mannose, glucose and cellobiose.

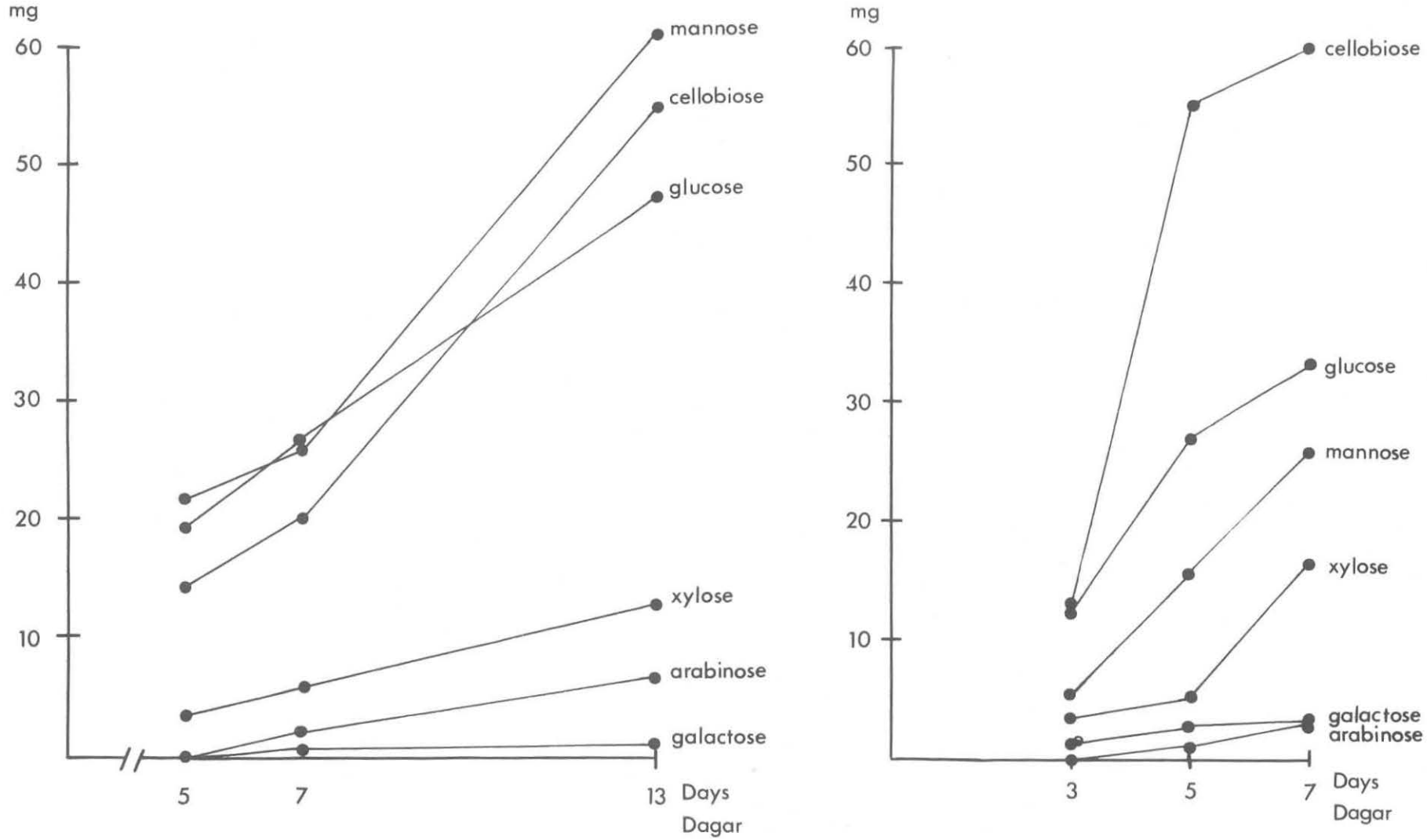


Fig 4. Mycelial production (dry weight) of P-B1 growing in a complete nutrient solution with various carbon sources. Incubation performed with (left) and without (right) shaking. Correction has been made for the weight of the inocula.

*Mycelproduktionen (torrvikt) hos B-B1 växande i en fullständig närlösning med olika kolkällor. Odlingarna har gjorts med (t v) och utan (t h) skakning. Korrektion för ympmaterialets vikt har gjorts.*

## 4. PRODUCTION OF CELLULASE, XYLANASE AND PHENOL OXIDASES

The enzyme production was tested on Avicel and birch wood meal. The fungus was cultivated in 100 ml flasks with 100 mg of the substrate and 30 ml nutrient solution of the following composition: ammonium sulphate 0.5 gm; L-asparagine 0.5 gm; potassium dihydrogen phosphate 1.0 gm; potassium chloride 0.5 gm; magnesium sulphate 0.2 gm; calcium chloride 0.1 gm; yeast extract 0.5 gm and 1000 ml of de-ionized water. This medium was used together with cellulose and agar by Eggins and Pugh (1962) for the preparation of cellulose agar plates.

The flasks were inoculated with a suspension of mycelium and placed in a rotary shaker at 200 RPM. The temperature was 25 - 26°C. After three weeks the mycelium and the remains of the substrates were filtered off, dried at 105° overnight and weighed. The true weight loss of the substrate could not be determined due to the mycelium present with the remaining substrate. The data obtained by simply calculating the weight loss as the difference between the dry weight of added substrate and the dry weight of remaining substrate + mycelium give a rough idea of the degradation of the substrate. Using this method, the weight loss of Avicel was 40.7 percent whereas the birch wood meal had only lost 3.9 percent.

The culture filtrates were then used for testing the presence of cellulase and xylanase. The test were carried out according to a method described by Stranks and Bieniada (1971) where small droplets of culture filtrates are added to a monolayer of cellulose or xylane in agar. The formation of clear zones around the droplets indicates cellulase or xylanase activity. We used 0.25 percent of Walseth cellulose in the top layer instead of the suggested 2 percent. Larch xylan (Koch-Light Laboratories Ltd) was used in 2 percent concentration in the top layer of the plates for testing xylanase.

40 µl from each of the filtrates were added to both types of testing plates. After incubation of the plates at 40°C for 72 hours, the diameter of the clear zones were measured. The filtrate from the flasks with Avicel gave a clear zone of 7 mm on the cellulose plates

and 15 mm on the xylan plates. The corresponding values for the filtrate from flasks with birch wood meal was 5 mm and 10 mm.

Thus, it is evident that the fungus produces both cellulase and xylanase. The amount of enzymes in the culture filtrate is greater on Avicel than on birch wood meal under the conditions used here.

Drop tests were performed according to the method described by Käärik (1965). Strong colour reactions were obtained with α -naphthylamine, o-anisidine and 2,5-xylidine No reactions occurred with p-cresol and tyrosine. Thus, P-B1 would belong to the group III of decay fungi according to the system set up by Käärik (1965). Fungi in group III produce laccase but no tyrosinase.

## 5. WOOD DECOMPOSITION

Cubical (1 x 1 x 1 cm) blocks of birch sapwood were used. The dry weight of the blocks was determined after drying at 105°C. They were then sterilised and placed on vermiculite cultures of P-B1 according to "Method 2" and "Medium B" described by Henningsson (1967). Five blocks were placed in each flask. After periods ranging from one to five months at approx. 22°C, five flasks were withdrawn. The blocks were weighed immediately after withdrawal and after drying at 105°. The weight losses for the 25 blocks were determined and an average weight loss was then calculated for each decay period.

The results are presented in table 1 and fig 5. Within the experimantal period used here, the decay measured as the average weight loss of the blocks proceeded at a fairly constant speed. However, the variation in weight loss between individual blocks increased with time. The moisture content of the blocks remained at about the same level throughout the course of decay. Substantial variations were not registered until after four months e.g. when one block that had lost more than 80 % of its weight had a moisture content of 140 %.

Table 1. Weight losses and moisture contents of birch wood blocks decayed by the Basidiomycete P-B1 according to the vermiculite method.

*Substansförlust och fuktkvoter hos klotsar av björksplint angripna av Basidiomyceten P-B1 enligt vermiculitmetoden.*

| Incubation period, months *Rötnings-period mån.* | Weight loss in percent of the original dry weight *Substansförlust i % av den ursprungliga torrvikten* | Moisture content in per cent of the dry weight after incubation *Fuktkvot i % av torrvikten efter rötning* |
|---|---|---|
| 1 | 14.9 (12.1 - 18.5)[1)] | 80 (72 - 96)[1)] |
| 2 | 25.2 (19.7 - 32.7) | 85 (73 - 117) |
| 3 | 36.5 (24.2 - 57.1) | 77 (57 - 106) |
| 4 | 45.6 (34.9 - 80.7) | 79 (53 - 140) |

[1)] Minimum and maximum values within brackets.

*Minimum- och maximumvärden inom parentes.*

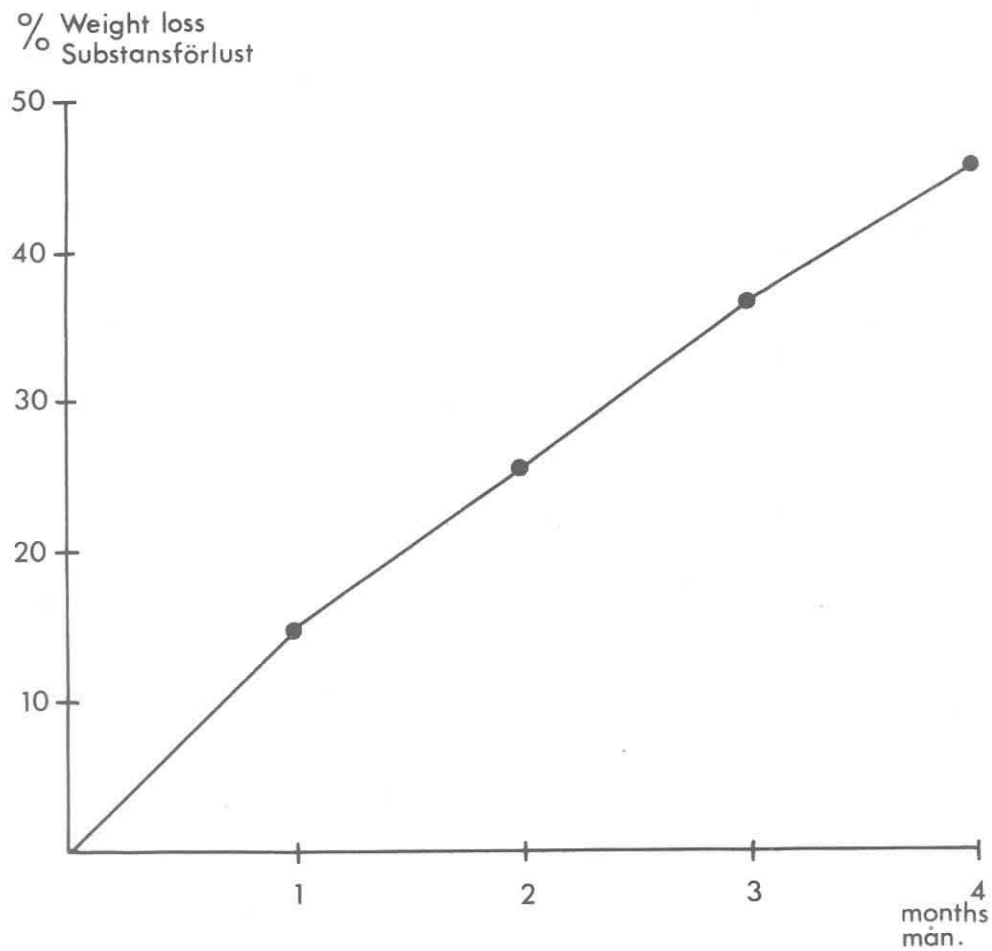


Fig. 5. Progressive changes in average weight loss of birch sapwood (1x1x1 cm) blocks attacked by P-B1 growing on vermiculite cultures.
*Fortlöpande förändringar i substansförlust hos klotsar (1x1x1 cm) av björksplint angripna av P-B1 växande på vermiculitkulturer.*

It must be borne in mind that this decay experiment was performed at temperatures nearly 15 centigrades below the optimum for growth. Therefore P-B1 must be regarded as a very active wood-destroying fungus. In that respect P-B1 can be juxtaposed to other known white rot fungi such as *Polyporus versicolor*, *Polyporus hirsutus*, *Polyporus adustus*, etc. (Henningsson 1967).

The blocks decayed as described above were ground and analyzed for cellulose and lignin. The lignin analyses were made according to a method including hydrolysis in 72 % sulphuric acid described in Technical Information CCA5 from the Swedish Association of Pulp and Paper Engineers. The cellulose content was analysed according to Seifert (1956). The method employs the use of acetyl acetone and hydrochloric acid and gives a cellulose almost completely free from hemicelluloses.

The results can be seen in Table 2 and fig 6. There it is quite clearly illustrated that the chemical pattern of wood decomposition was significantly different from that of ordinary white rot fungi (Campbell 1930, 1931 and 1932, Cowling 1961, Seifert 1968, Henningsson 1967). In an attack by white rot fungi, the decomposition of lignin and cellulose normally proceed simultaneously and at a rate which is proportional to the percentual occurrence of these substances in the wood. The proportion between cellulose and lignin does not change significantly during most of the course of decay. When P-B1 grows on malt extract and is allowed to attack birch wood, however, the attack starts with a pronouced decomposition of the lignin fraction. When analyzing the wood, this was demonstrated by the reduction in hardly soluble lignin amounting to nearly 50 % at a weight of only 15 %. At the same weight loss level, the original amount of cellulose had decreased by only two percent. However, the more the decay proceeded, the more the attack on the cellulose accelerated. At weight losses of 45 - 50 % the same percentage of the cellulose had also been broken down.

If the percentage weight loss and the percentage of cellulose and lignin are totalled for a certain stage of decay, a percentage of rest substances can be calculated. This fraction includes i.a. hemicelluloses and easily soluble lignin. The decomposition rate of this fraction remained at approximately the same level during the course of decay.

Table 2. Contents of cellulose, lignin and the rest fraction in birch wood decomposed to various weight losses by the white rot fungus P-B1.

*Cellulosa - lignin och resthalt hos björkved rötad av vitröte-svampen P-B1 till olika substansförluster.*

| Weight loss *Substans-förlust* | Content based on weight of decayed wood *Halt beräknad på rötade vedens vikt* | | | Content based on weight of sound wood *Halt beräknad på friska vedens vikt* | | | Relative content *Relativ halt* | | |
|---|---|---|---|---|---|---|---|---|---|
| | lignin % | cellulose % | rest % | lignin % | cellulose % | rest % | lignin % | cellulose % | rest % |
| 0 | 18.3 | 44.8 | 36.9 | 18.3 | 44.8 | 36.9 | 100.0 | 100.0 | 100.0 |
| 14.9 | 10.9 | 51.6 | 37.5 | 9.3 | 43.9 | 31.9 | 50.7 | 98.0 | 86.4 |
| 25.2 | 10.7 | 53.0 | 36.3 | 8.0 | 39.6 | 27.2 | 43.7 | 88.4 | 73.7 |
| 36.5 | 10.4 | 53.5 | 36.1 | 6.6 | 34.0 | 22.9 | 36.1 | 75.9 | 62.1 |
| 45.6 | 11.2 | 51.5 | 37.3 | 6.1 | 28.0 | 20.3 | 33.3 | 62.5 | 55.0 |

In the earlier stages of decay, e.g. at a weight loss of 15 percent, approximately 14 percent of the rest fraction had been decomposed. These results give reason to assume that hemicelluloses are also decomposed in the early stages of decay simultaneously with the lignin decomposition.

## 6. ATTEMPTS TO OBTAIN BIOLOGICAL DEFIBRATION IN BIRCH WOOD CHIPS

Birch wood chips, ca 20 x 20 x 2 mm, were cut from air-dried, sound sapwood of *Betula verrucosa*. The chips were divided into six portions which were vacuum impregnated with water solutions of the following compositions:

a) distilled water
b) 0.5 % $(NH_4)_2\ NO_3$
c) 1.0 % Asparagine
d) 1 % Casein hydrolyzate
e) Medium B; glucose excluded (a complete nutrient solution c.f. Henningsson 1967 page 8)
f) 2.0 % Malt extract

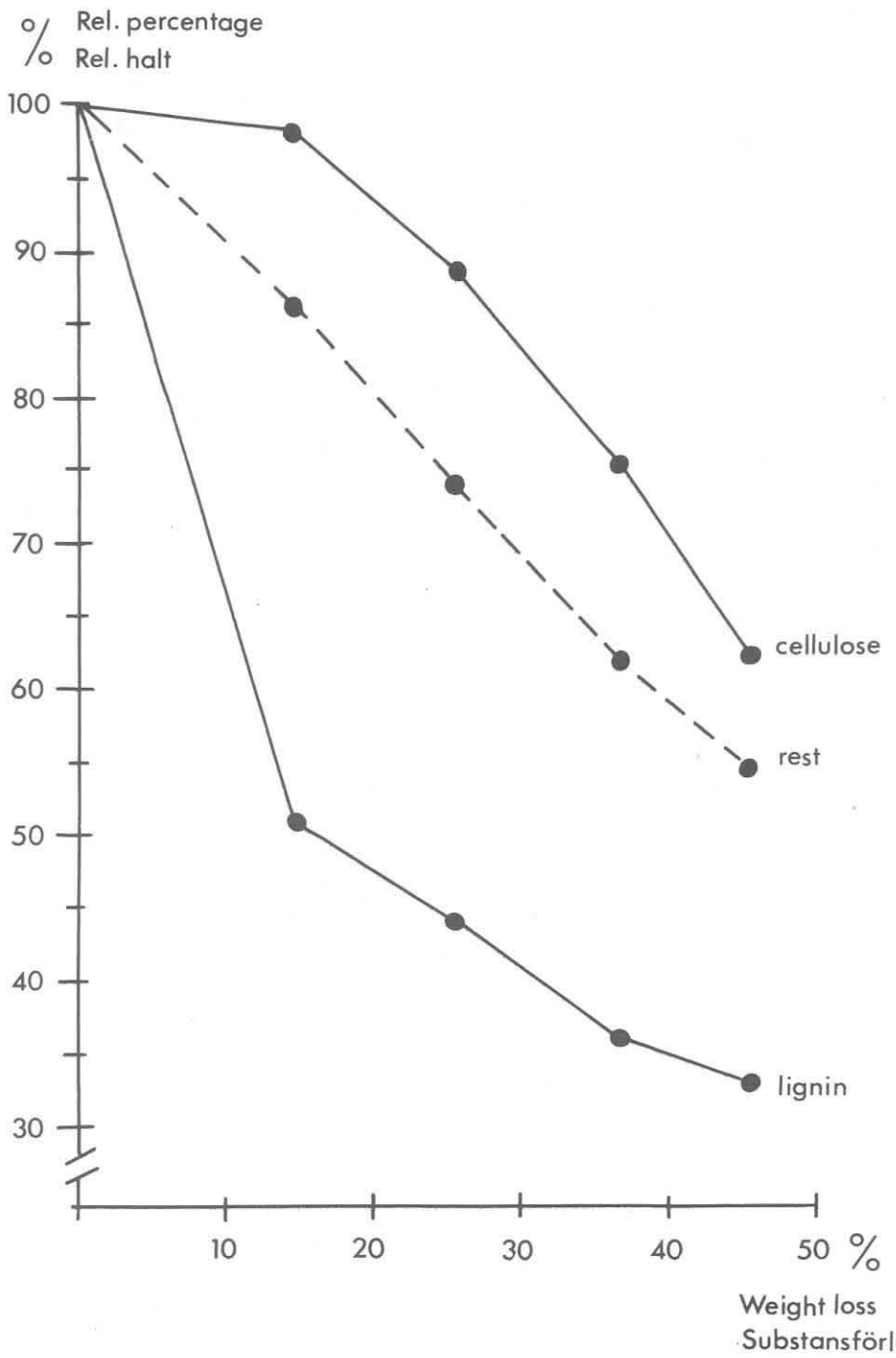


Fig 6. Progressive changes in the relative percentage of cellulose and lignin in birch sapwood attacked by P-B1. A rest fraction containing easily soluble lignin and hemicelluloses has been calculated theoretically.

*Fortlöpande förändringar i den relativa halten av cellulosa och lignin hos splintved av björk angripen av P-B1. En restfraktion innehållande lättlösligt lignin och hemicellulosor har teoretiskt beräknats.*

After impregnation the chips were allowed to dry on an absorbant paper for approximately 15 minutes. Portions of about 20 g of wet chips were placed in Erlenmeyer flasks. Four flasks of each type of chips were prepared. After autoclave sterilization the flasks were inoculated with the fungus P-B1.

The flasks were incubated at room temperature (21 - 23°C) for periods ranging from one to five months. During the incubation period 5 ml of sterile distilled water was added twice, one month and two and a half months after the inoculation.

Flasks were withdrawn after one, two, three and five months. A few chips from each flask were collected for microscopic and enzymatic investigations. The remaining chips were, after the addition of 100 ml water, submitted to a two minutes mechanical treatment in a MSE Ato-Mix homogenizator at highest speed. The treated sample was then suspended and washed in approx. 1.5 l of water and filtered in two steps using: 1) a 2 mm metallic sieve, 2) filter paper Munktell No 50. The last filtration was made in a Büchner funnel using a water suction pump. The material collected at each filtration level was dried, weighed and examined, especially with regard to the degree of defibration and the felting tendency.

The wood chips were also examined microscopically with regard to the type of attack. The presence in the chips of cellulases and xylanases was demonstrated by the use of a modification of the method described by Stranks & Bieniada (1971).

After the incubation all chips had been bleached to some extent, except for those to which ammonium nitrate had been added. Loose fibers could be scraped off the surface of the chips pre-treated with water, asparagine or malt extract.

Thin cross-sections were cut, stained with safranin and examined under a light microscope. Some loose fibers scraped from the surface of the chips were examined in polarized light. Following microscopical observations were made in relation to the various pre-treatments:

a) Water

Two clearly different types of attack were observed in these chips. The first type of attack was cell wall thinning which started from the $S_3$ layer and proceeded outward until approximately half of the $S_2$ layer was removed. The attack never reached the middle lamella. The $S_3$ layer was evidently broken down since no remnants of it were seen in cells where the $S_2$ layer was attacked. The cell wall adjacent to the cell lumen in attacked cells was smooth, indicating a uniform degradation of the $S_2$ layer.

The second type of attack also started from the $S_3$ layer. The first sign of attack was a narrow band around the lumen that did not stain with safranin. Later, a slight swelling of this part of the cell wall was apparent. The swelled parts that did not stain with safranin stained faintly blue with zinc chloro-iodide solution and blue with Astrablue.

It was concluded that these reactions were due to delignification of the cell wall. The delignification proceeded toward the middle lamella which was eventually degraded. The thickened area of the middle lamella at the cell corners was the last part to be degraded.

The fibers sometimes separated even before the middle lamella was completely degraded. This was certainly due to the stresses arising during the sectioning of the wood. Most of the swelled and separated fibers showed no signs of cellulose degradation but when scrapings of fibers from the chip surfaces were examined in polarized light, strong attack of the cellulose was observed in some of the fibers.

There was a certain separation of the two types of attack within the chips. Most of the cell wall thinnings occurred in the interior of the chips, whereas the delignification seemed to have started at the surface and proceeded inward.

b) Ammonium nitrate 0.5 %

Rather extensive cell wall thinnings, sometimes extending to the middle lamella region, were observed. The middle lamella could not be seen to be degraded. In the early attack the cell wall adjacent to the lumen was smooth but became more irregular as the attack advanced.

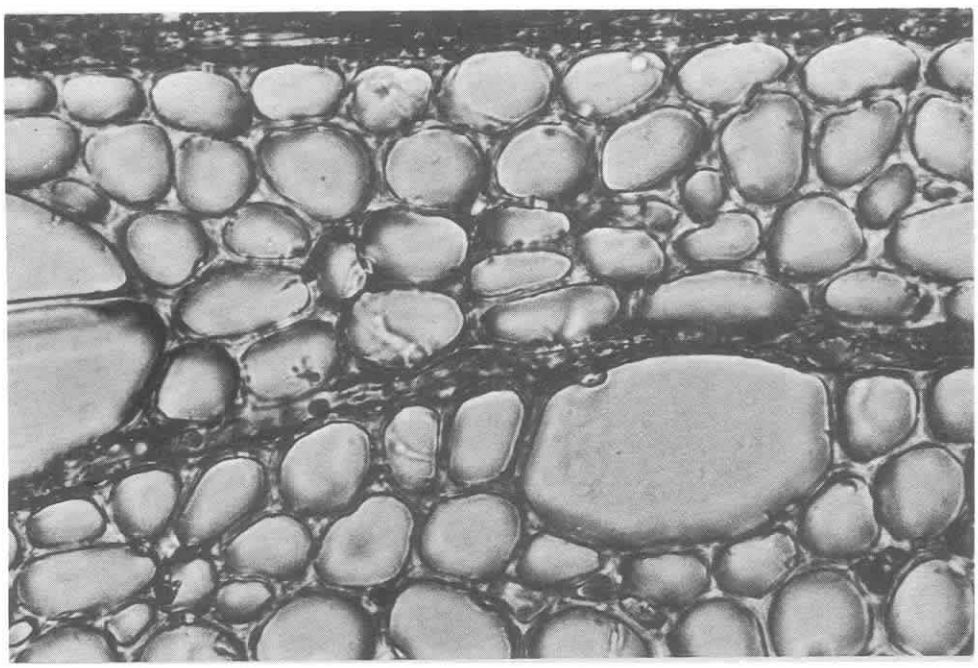

Fig 7. Birch wood chips impregnated with 1.0 % asparagine. Cross section showing cell wall thinnings.

*Björkflis impregnerad med 1.0 % asparagin. Tvärsnitt med cellväggsförtunningar.*

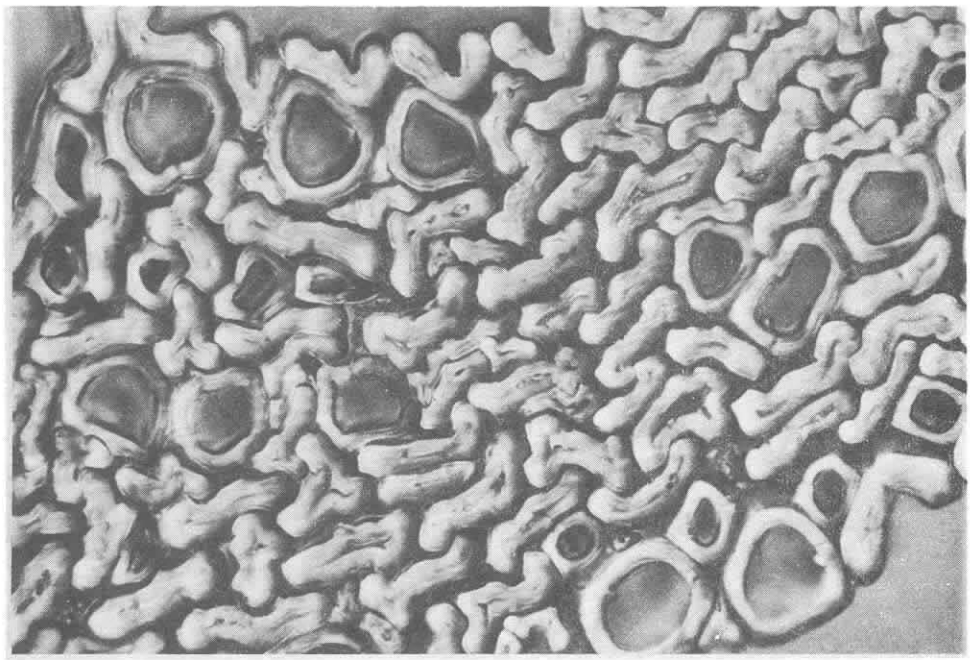

Fig 8. Birch wood chips impregnated with 1.0 % asparagine. Cross section showing advanced delignification of the wood.

*Björkflis impregnerad med 1.0 % asparagin. Tvärsnitt med långtgående delignifiering av veden.*

The delignification type of attack was not observed in these chips.

c) Asparagine 1.0 %

The attack in these chips was similar to that in the chips with only water. However, the attack was stronger and the delignification type clearly dominated. The cell wall thinnings were smooth and proceeded to the middle lamella (fig 7) which occasionally was also degraded. The delignification type of attack also occurred in the interior of these chips, where most of the fibers separated after swelling and degradation of the middle lamella. This occurred apparently without degradation of the cellulose (fig 8). Some fibers separated before any apparent swelling, evidently due to some changes in the middle lamella region that could not be observed in the light microscope. Separating cells with cell wall thinnings were also observed.

Strong attack on the cellulose was seen in some fibers scraped from the surface of the chips when viewed in polarized light.

d) Casein hydrolyzate 1.0 %

Smooth cell wall thinnings occurred but they were only slight and more than half of the $S_2$ layer remained. Delignification also occurred, extending to the middle lamella region, but the middle lamella did not appear to have been attacked. No separation of fibers was observed. The two types of attack seemed to occur simultaneously in several cells since severe attack on the cellulose was observed in many of the swollen cells.

e) Medium B without glucose

Very irregular cell wall thinnings were found in these chips (fig 9). The attacks were localised to small parts of the cell wall. These attacks occasionally proceeded to the middle lamella which was also degraded. Delignification occurred but degradation of the middle lamella could not be observed. Very few of the fibers separated. The two types of attack also occurred simultaneously in several cells.

f) Malt extract 2.0 %

Smooth cell wall thinnings occurred, extending to the middle lamella region, but the middle lamella was not degraded. Delignification

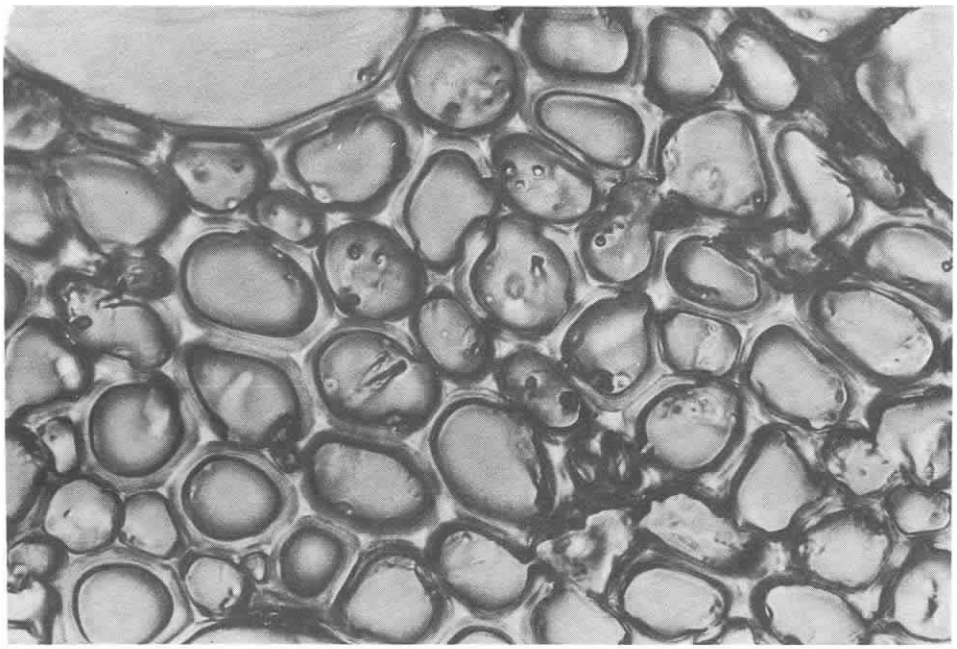

Fig 9. Birch wood chips impregnated with Medium B. Cross section showing very irregular erosion of the cell walls.

*Björkflis impregnerad med Medium B. Tvärsnitt med mycket oregelbunden cellväggserosion.*

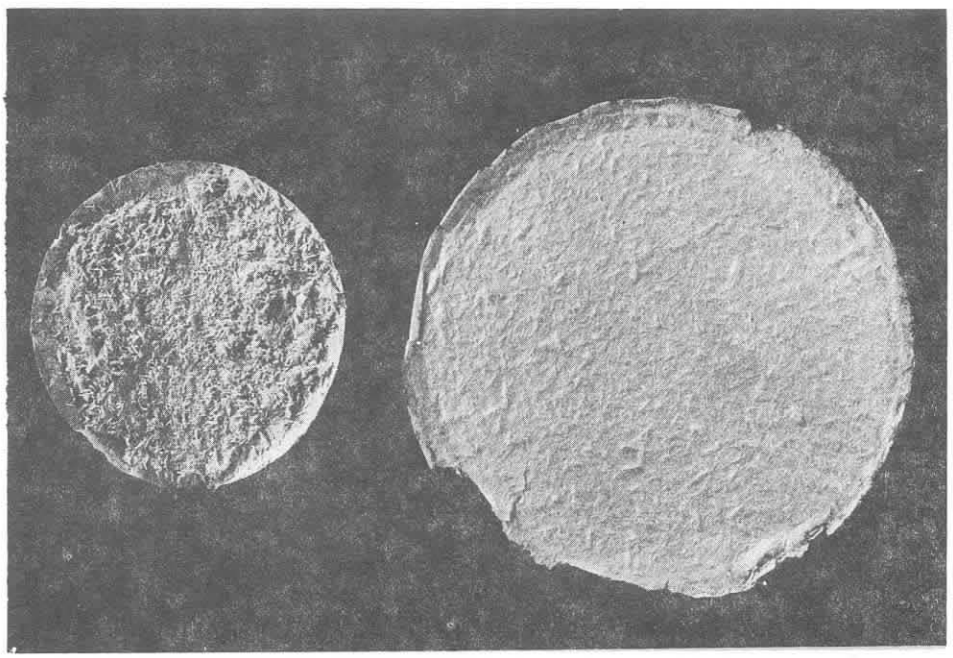

Fig 10. Mechanically treated material collected at the second filtration level from wood pre-treated with ammoniumnitrate (left) and asparagine (right). Note that the pre-treatment with ammoniumnitrate yielded only separate wood particles (saw dust) while pre-treatment with asparagine gave a real sheet of paper.

*Material från andra filtreringssteget. Veden har förbehandlats med ammoniumnitrat (t v) och asparagin (t h). Observera att behandlingen med ammoniumnitrat endast resulterade i separata vedpartiklar (sågspån) medan förbehandling med asparagin verkligen gav ett pappersark.*

involved the $S_2$ layer and the middle lamella which was observed to be broken down. Several of the fibers separated. The delignification showed a marked tendency to proceed along the rays.

The results of the birch chips experiments are summarized in table 3. The table clearly shows that the different pre-treatments of the chips exerted different influences on the enzymatic activity and the type of attack in the wood, as well as on the pulping properties of the wood after treatment in the homogenizator.

With respect to the pulping tendency after treatment in the homogenizator, it was quite obvious that the best defibration and felting tendency was attained in wood pre-treated with asparagine. See fig 10, 11 and 12. Practically no defibration or felting tendency could be observed when ammonium nitrate was used.

Diffusable cellulase and xylanase could be demonstrated only in chips treated with ammonium nitrate and casein hydrolyzate.

## 7. DICUSSION

For a rather long time there existed, especially among people within the pulp industry, a false conception concerning white rot. Because of the bleaching of the wood, it was supposed that almost only lignin was decomposed by white rot fungi. Consequently, if not too advanced white rot was almost of advantage for pulping, since a delignifying process had already started. Despite an increasing number of reports showing that cellulose is attacked to the same degree as lignin in white rotted wood, the false conception was still heard.

As a result of the simultaneous attack on the major wood components, the relationship between lignin and cellulose changes very little during the course of white rot attack. This also contributed to confusion about the real nature of white rot, since the pulp yield loss was very small when calculated on the weight of the wood fed into the digestor. If the pulp yield was calculated, however, on the weight

Fig 11. Microscopic surface picture of the paper sheet obtained from wood pre-treated with asparagine.

*Mikroskopisk bild av ytan hos det pappersark som erhölls när veden förbehandlats med asparagin.*

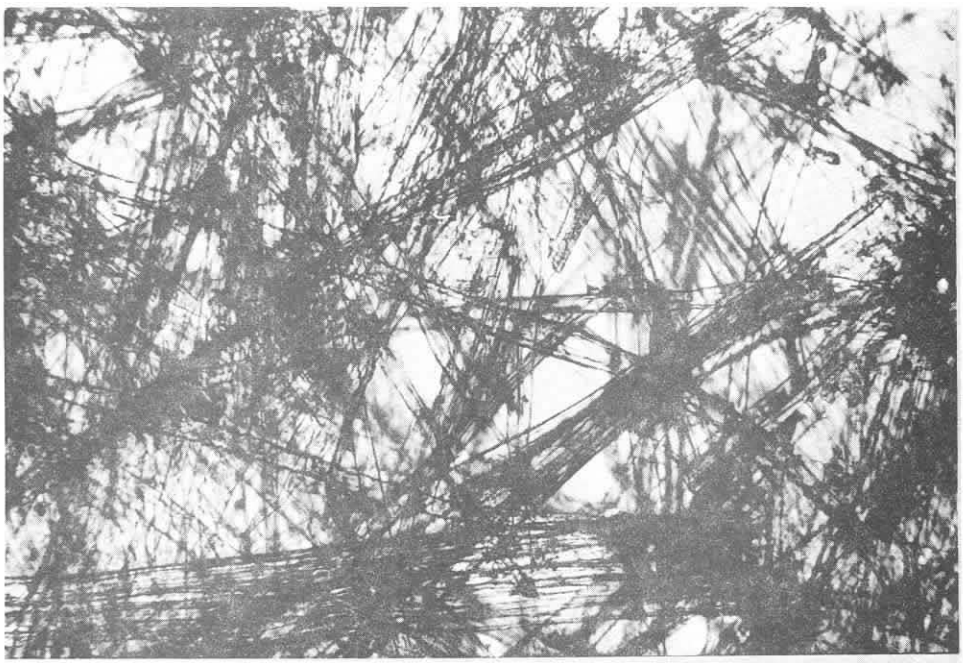

Fig 12. Microscopic picture of the fibers of a loosely packed part of the paper obtained from wood pre-treated with asparagine.

*Mikroskopisk bild av fibrer från ett löst packat parti av det papper som erhölls när veden förbehandlats med asparagin.*

Table 3. Examination of birch chips attacked by P-B1 during 1 - 5 months at room temperature

*Undersökning av björkvedsflis angripen av P-B1 under 1 - 5 månader vid rumstemperatur.*

| Additive<br>*Tillsatser* | Enzyme activity<br>*Enzymaktivitet* | | Microscopic observations<br>*Mikroskopiska observationer* | | | Observations of defibration and tendencies toward paper formation<br>*Observationer av defibrering och pappersbildningstendenser* |
|---|---|---|---|---|---|---|
| | cellulase | xylanase | thinning of the cell wall<br>*cellväggsförtunning* | delignification<br>*delignifiering* | other observations<br>*övriga observationer* | |
| Water | 0 | 0 | + | ++ | | Relatively good defibration; good felting<br>*Relativ god defibrering; god filtning* |
| Ammonium nitrate | + | + | ++ | 0 | | Insignificant defibration; felting absent, brownish discolouration<br>*Obetydlig defibrering; ingen filtning; brunaktig missfärgning* |
| Asparagine 1.0 % | 0 | 0 | +++ | +++ | Delignification without swelling of $S_2$<br>*Delignifiering utan svällning av* $S_2$ | Good defibration; good felting<br>*God defibrering; god filtning* |
| Casein hydrolyzate 1.0 % | + | + | + | + | | Weak defibration; insignificant felting<br>*Svag defibrering; obetydlig filtning* |
| Medium B without glucose | 0 | 0 | ++ | + | | Slight defibration; insignificant felting; granular<br>*Någon defibrering; obetydlig filtning; kornig* |
| Maltextract 2.0 % | 0 | 0 | ++ | ++ | | Relatively good defibration; felting; however, a substantial part of greater particles and gelatineous material<br>*Relativt god defibrering; filtning; dock stor andel större partiklar och gelantinartat material* |

of the wood before white rot attack, the percentual pulp yield losses proved to be of the same order as the percentage weight loss of the wood (Henningsson 1967).

It is now generally accepted that white rot fungi normally decompose the major wood components simultaneously and in proportion to their occurrence in the wood. In recent years there has even been a tendency to over estimate this generalization and to classify wood-decomposing organisms into rigid systems based on chemical analyses of the attacked wood (Seifert 1968). To our knowledge, at least one white rot fungus deviates from this general scheme to a certain degree - *Trametes pini* (Thore) Fr. In the incipient stages of decay this species decomposes the lignin faster than the cellulose. In the excellent work of Meier (1955), *Trametes pini* was further shown to destroy the middle lamella extensively, thus causing a kind of defibration of the wood. This type of attack was also found for *Fomes annosus* (Fr.) Cooke in birch wood, whereas the cellulose and lignin in spruce wood were degraded simultanously.

The potential possibility of using the fungus P-B1 as a "wood defibrator" was discovered more of less by change. However, the advantages of this species very soon became evident. As a result of its fast radial growth (Fig 1), the wood is colonized very rapidly. In combination with the rather high optimum temperature, this makes P-B1 competitive if the temperature is maintained between 30 and 40°C.

Microscopical observations revealed that the fungus often preferentially attacked the middle lamella region. The results of chemical analyses of the attacked wood showed that in a weight loss of up to 15 percent practically no cellulose was decomposed, whereas nearly 50 percent of the lignin had been broken down. In combination, these observations strongly supported a positive prognosis with respect to the use of P-B1 as a "wood defibrator".

However, it was also quite clear that the cellulose of the wood fibres was attacked, especially when the incubation time was extended. If the attack was allowed to proceed beyond 15 percent weight loss of

the wood, the cellulose break-down increased rapidly (fig 6). Possible ways of avoiding or reducing these disadvantages had to be studied. Since P-Bl does not produce spores or oidia in culture as does *Polyporus adustus*, which was used by Eriksson & Godell, it was considered to be less suitable for mutagenic treatment. Other types of manipulations were needed.

Liese and Ammer (1964) observed that the moisture content of the wood influenced the shape of the cavities formed by soft rot fungi. Bergman & Nilsson (1968) found that the temperature influenced the type of attack obtained in birch wood by a thermophilic soft rot fungus. Several observations of soft rot as well as white rot fungi (Nilsson, not published) indicate that the type of wood attack is, to a certain degree, regulated by factors like wood species, temperature, accessible nitrogen, type of nitrogen and accessible carbohydrates. A strain difference within the same species may also exist. The fact that the type of wood attack by a certain fungus is not fixed but may vary considerably due to different methods for decay tests is a possible explanation of the diverging results reported for some fungi. For example, Jahn et al. (1963) give an account of the conflicting reports on wood decay by *Polyporus versicolor* (L. ex Fr.) Fr.

In this investigation the type of nitrogen supply to the wood seemed to be of special interest. It might be noted that asparagine, which gave the best defibration, can not only be used as a nitrogen source but also as possible alternative carbon source. From our observations it is proposed that it might be possible to actively control and direct the type and extent of wood decomposition carried out by P-Bl. As is demonstrated in chapter 6, we succeeded in turning the decay process of P-Bl in the desired direction especially by manipulation of the nitrogen source.

However, the factors influencing the type of attack must be studied much more intensively before any conclusion can be reached regarding the possibility of controling a process of fungal wood defibration by changing the physiological environment.

There are indications that other fungi might also be suitable for defibration purposes. A thorough investigation in order to find potential "defibrator organisms" is therefore highly desirable The whole range of measures (genetical as well as physiological) which might be used to change, control and direct the wood attack must then be considered.

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SAMMANFATTNING

Samtidigt med undersökningar av vedangripande mikroorganismer i Östersjön bedrevs ett "screening"-arbete i avsikt att finns svampar lämpliga för defibrering av ved. Därvid påträffades i nordligaste Östersjön en basidiomycet (P-B1) med utpräglad förmåga att defibrera björkved.

Svampen, som sannolikt tillhör Peniophora-släktet har optimal tillväxt vid +35°C och pH 5. Koksalt har så starkt hämmande inverkan på tillväxten att svampen kan betraktas som halofob.

Cellobios, glukos och mannos utgjorde goda kolkällor för P-B1. Galaktos kunde inte utnyttjas alls och tillväxten på xylos och arabinos var tämligen långsam.

Rötningsförsök med björkved visade att svampen orsakar en snabb vednedbrytning. Därvid angrips i början ligninet i mycket större utsträckning än cellulosan. Vid 15 % substansförlust hade hela 50 % av ligninet men endast 2 % av cellulosan nedbrutits.

Björkvedsflis, som förbehandlats med framför allt olika kväveföreningar inympades med P-B1. Mikroskopiska undersökningar av den angripna flisen visade därvid att angreppsbilden på vedfibrerna varierade med olika förbehandlingar. Impregnering med asparagin resulterade bl a i en kraftig delignifiering och defibrering. Ammoniumnitrat gav däremot enbart angrepp av erosionstyp och ingen defibrering.

Mekanisk bearbetning av den angripna flisen och fraktionerad filtrering visade att en god defibrering och klar tendens till peppersbildning (filtning) erhölls med vissa förbehandlingar (t ex asparagin). Impregnering med ammoniumnitrat gav ingen defibrering och ingen tendens till pappersbildning. Undersökningarna visade således att det, genom att förändra den fysiologiska miljön i veden, var möjligt att styra svampens angrepp på vedstrukturen.